63-4-2

PROGRESS REPORT

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THE EFFECT OF COMPAZINE ON THE METABOLISM OF THE GERBIL

DA-49-193-MD-2216

Qualified requestors may obtain copies of the report from ASTIA

Unclassified

1963

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INTRODUCTION

General

It has been known for some time that the environmental temperature may influence chemical and physical processes within an organism and that certain drugs and chemical compounds are altered in their action because of the environmental temperature (Fuhrman, 1946; Keplinger, Lanier, and Deichman, 1959; Shemano and Nickerson, 1958). This environmental temperature may influence the rate of absorption, rate of diffusion, distribution or metabolic breakdown of a compound. This phenomenon has been ignored in a practical way; for example, in the literature consulted there is no indication of altered drug dosages for people who live or work in extremes of environmental temperatures. As Keplinger et al. (1959) point out, "the effect of environmental temperature has not been given consideration in setting limits of tolerance for exposure to industrial chemicals."

Fuhrman and Fuhrman (1961) have noted that the use of hypothermia in medicine and surgery emphasizes the fundamental need for data on the mechanisms of drug action at various temperatures; of primary importance is the nature of the biochemical processes at these temperatures. They also point out that there have been few biochemical investigations of drugs at various temperatures, but that the investigations have been concerned largely with the practical applications of temperature differences and their effects on the action of drugs.

With the advent of tranquilizing, and psychotropic drugs in general, it becomes increasingly important that the mechanisms of action of these drugs be understood. Sidman's (1959) studies have shown that a definite interaction exists between drugs and behavior and the fact that behavioral patterns may change with various environmental temperatures should not go unnoticed. Thus, until proven otherwise, it might be wise to assume that the effects of therapeutic doses of drugs as well as maximum allowable concentrations of various chemicals are influenced in man by the environmental temperature, as emphasized by Fuhrman and Fuhrman (1961).

Drug of Interest

The drug used in this investigation was 2-chloro-10-(3-(1-methyl-4-piperazinyl)-propyl)-phenothiazine, generic name, prochlorperazine (See Appendix A). The trade name is Compazine; Compazine will be used to refer to the drug throughout this report. The reasons for choosing this drug were its availability and its known tranquilizing capabilities.

Compazine is a member of the phenothiazine group which automatically classifies it as a "major tranquilizer." (See Appendix B for a comparison of the major and minor tranquilizers). The pharmacological effects of the major and minor tranquilizers (Appendix C) show many similarities, as well as many differences. The major tranquilizers exert greater autonomic effects and have a tendency to produce tremors. Their most significant action is the capacity to depress the hypothalamus.

The minor tranquilizers differ among themselves with respect to their autonomic blocking activity. This activity is more characteristic of the diphenylmethanes, whereas the muscle relaxant action and multineuronal blocking drugs are largely of the diol-carbamate type.

Most of the tranquilizers now being introduced belong to the phenothiazine group. Drugs in this group possess a multitude of actions: they are known to block the adrenergic nervous system; they produce a sedation, hypnosis, and anesthesia; they block conditioned reflexes; and, they potentiate the action of analgesics and anesthetics. The general pattern of tranquilizers is to relieve psychotic patients of emotional tension, agitation, and excitement. Compazine is an agent that shares high potency and rapid effect with other members of this group. It is approximately four times as potent as chlorpromazine (Thorazine) (Benson and Schiele, 1962). Thorazine is the most widely used phenothiazine derivative and was the first phenothiazine to be introduced for general medical use.

Compazine occurs in two salt forms, namely, prochlorperazine dimaleate and prochlorperazine ethane disulfonate. The latter was used because it readily dissolves in distilled water; the former dissolved in water only in small amounts and with great difficulty.

Choice of Animal

The gerbils, *Meriones unguiculatus*, which were used in this experiment, were first identified by Milne-Edwards in 1867; this identification was later confirmed in a revision of the genus in 1947 (Chatworth-Musters and Ellerman, 1947). Milne-Edwards found these animals living on the barren and stony plains of Mongolia, North of China. On a current map, this area would correspond to northwest Manchuria near the Mongolian plateau. These small desert rodents are now found throughout North Africa, southwest Russia, Asia Minor, and southwest Asia (Robinson, 1959).

Milne-Edwards described these animals as having a long tail (according to Chatworth-Musters and Ellerman, it averages 91% of the head and body), a greyish-brown coat on the dorsal side, a whitish coat on the ventral side; and, its claws were dark and sturdy. Chatworth-Musters and Ellerman published the average measurements and extremes of nineteen skins of *Meriones unguiculatus*. Their results follow:

PART	MEAN (mm.)	RANGE (mm.)
head and body	111	100-125
tail	102	96-110
hind foot	27	26-30
ear	13	12-15

In their natural habitat rodents of this genus dig elaborate underground retreats. These retreats are often at two levels and include numerous chambers for both storage and nesting. These chambers are interconnected with a labyrinth of runways. The natural diet of this animal probably consists largely of a dry-grain diet. The exact diet of this genus could not be found in the literature.

It has been known for some time that desert mammals secrete a very concentrated urine, twice as concentrated as sea-water, with respect to electrolytes (Schmidt-Nielsen, 1950b). The fact that desert mammals may live without water for considerable periods of time has been attributed to (1) elongated tubules within the kidney, (2) nocturnal habits, and (3) the humidity present within the burrows. Vimtrup and Schmidt-Nielsen (1952) have demonstrated that both the distal tubules and collecting tubules are significantly different in the kangaroo rat as compared to the albino rat. In the kangaroo rat the distal and collecting tubules are much larger and different in structure from those of the white rat, while the glomeruli and proximal tubules resemble each other closely. Since the animals are largely nocturnal, they face less chance of losing water through evaporation from the lungs. Schmidt-Neilsen and Schmidt-Nielsen (1950b) have shown that the higher humidity within the burrows reduces evaporation from the lungs to approximately 75 per cent of what it normally is when the dry desert air outside the burrow is respired. All of these factors combined add to the water economy of the gerbil and to an extent account for its capability of going without water for considerable periods of time.

The animals were chosen for this experiment primarily because of their availability and because of their uniqueness. Little experimental work has been done on them, as indicated by the lack of available literature. They are characterized by their ease of handling and care; offensive odor is at a minimum. One may handle these animals without fear of being bitten, although they may attempt to dig through one's hand. Little care is required as the feces are very dry; urination is seldom, and of small volume. Since the gerbils conserve water, the refilling of water bottles every day (necessitated by many laboratory animals) is obviated.

Previous Work

Much work has been done with regard to measuring the oxygen consumption in small animals. Pearson (1947) studied the basal metabolic rate of sixteen species and subspecies of small mammals ranging from the shrew to the flying squirrel. He found that each genus consumed oxygen at a rate characteristic of that genus and concluded that each has its own metabolic "personality." He also found that there was a daily metabolic cycle corresponding with the activity cycle of the species. Morrison (1948) did a similar study on small wild mammals over a 24-hour period and noticed that the per cent increase in oxygen consumption during this active period or cycle is a convenient numerical index of the intensity of the 24-hour rhythm. From these average daily rates of oxygen consumption one may obtain some idea as to the energy utilization of the animal and therefore estimate the minimum food requirements.

Harvey (1958) performed experiments on oxygen consumption with guinea pigs and found that these animals were most suitable for assays because "even stimulation by dinitrophenol caused little distrubance to their natural quietness." Lyman (1948) measured the oxygen consumption and temperature regulation of hamsters. He observed that many factors could increase oxygen consumption and he especially noted that the size of the metabolic chamber might influence oxygen consumption. He noted that a smaller metabolism chamber increased oxygen uptake which he accounted for by saying that it made the animal more nervous.

A considerable amount of literature describes various aspects of oxygen uptake in the rat. Benedict and Petrik (1930) did metabolism studies with the wild rat and found that when the wild rat was acclimatized to domestic conditions, it had a tendency to assume the nature of the domesticated rat, with respect to oxygen consumed, as the period of captivity lengthened. He also reported that, in a number of laboratories, it was observed that as the temperature decreases, the metabolism increases by about 5 per cent with each degree centigrade decrease in temperature.

Adolph (1950) and Schwabe, Emery, and Griffith (1938) performed similar experiments in which they observed the effects of low temperatures on the metabolism of the rat. Adolph studied colonic temperatures between 26° C. and 16° C. and found that the oxygen consumed during cooling usually decreased linearly with the colonic temperature. They noted that when the colonic temperature was held constant over a period of one to three hours, no consistent change of oxygen consumption occurred. They concluded that the relation of oxygen consumption to colonic temperature is sufficiently fixed as it is not modified by cold acclimatization, but "it seems probable that the production of heat is more closely correlated with the surface temperatures than with the deep temperature."

It was observed by Schwabe et al. (1938) in their study with rats that when age and sex are accounted for there may be a difference in metabolic rate depending upon how it is expressed. They found that the metabolism of the 60-day males is approximately 10 per cent greater than their litter-mate sisters when expressed per unit of body surface; but when expressed per unit of body weight the difference is negligible. Similarly, Kleiber, Smith, and Chernikoff (1956) studied the metabolic rate of female rats with regard to age and body size. They agree with Schwabe et al. and state that "the effect of age on the metabolic rate can be expressed only in relation to a given unit of body size: per rat, per kilogram weight, etc." They found in their studies that the metabolic rate per rat increases with age as does body weight. They accounted for this increase in metabolism with age as a result of the rat's failure to regulate temperature. Conrad and Miller (1956) explain the increase in metabolic rate with age as a result of a decrease in the proportion of metabolically inert extracellular fluid, and an increase in the protein concentration of the fat-free body. They note that these changes would be expected to be associated with an increase in metabolic rate with age.

Davis (1937) in his study on the effect of advancing age on the oxygen consumption of rats disagrees with the foregoing conclusions. He maintains that Benedict's report of increase in metabolism with advancing age stems from the fact that sleep predominates in a younger animal while in an older individual, wakefulness predominates. Thus, one would expect an increase in metabolism with age.

Closely associated with the present work is that performed by Robinson (1959) who measured the oxygen consumption of Meriones unguiculatus at various environmental temperatures (15°-40° C.) and found that the oxygen uptake increased with a decrease in temperature below thermal neutrality according to the equation: ml. of O_2 = 5.660-0.141° C. He also observed that this genus has a greater capacity for temperature regulation under hot conditions (35°-40° C.) than does the kangaroo rat or the antelope ground squirrel. Robinson and Henrickson (1961) reported that Gerbillus pyramidum, another genus of gerbil, has a metabolic pattern similar to the albino rat. In both, the critical temperature is about 30° C. with a very narrow zone of thermoneutrality. This situation is in sharp contrast to Meriones unguiculatus which has a thermoneutrality range from 30°-40° C. Robinson and Henrickson account for this difference between the two gerbils as simply the superior capacity of M. unguiculatus to regulate its body temperature in a hot environment.

The present problem is concerned with oxygen consumption under the influence of a tranquilizer with the following items to be considered: (1) to ascertain the effects of pure oxygen (100%) on the metabolic rate; (2) to observe the drug effect on the metabolic pattern at various concentrations and temperatures; (3) to note whether the general metabolic rate has an influence on the action of the drug over a definite length of time; and, (4) to observe whether the temperature is the dominant factor in regulating metabolism or whether the drug exerts the dominant effect. The main purpose of performing the study was to throw further light on drug concentration-temperature interaction and to observe the effect of a tranquilizer on oxygen consumption.

Moreover, according to Herrington (1940), it is much more difficult to assemble from the literature reliable information on the effect of temperature below the area of thermal neutrality. "The increasing use of temperature extremes in conjunction with endocrine research, fever therapy, tumor growth, and related problems has given a new usefulness to such information for many studies in which the smaller animals are suitable reagents."

MATERIAL AND METHODS

Forty specimens of Meriones unguiculatus were used in the measurement of oxygen consumption. Their weights ranged from 51.0-108.0 gms. with a mean weight of 72.6 gms. The population was approximately evenly divided between the sexes. The animals were housed individually during the course of the experiment. The housing conditions were made as natural as possible by placing sand and nesting material in each cage. The gerbil diet consisted largely of sunflower seeds and "Purina lab-chow" pellets. Water was available *ad libitum*. Under these conditions the animals appeared healthy and had well-groomed pelages.

Oxygen consumption was measured by the closed-circuit method. Two different temperatures (18° and 26° C.) were used in these determinations. The instrument used was the Minute Oxygen Uptake Spirometer, model #160-144-B, Custom Engineering and Development Company, St. Louis, Missouri. (See Appendix D for a general description of the instrument). For each test made the gerbil was kept in the respiratory chamber for three hours. The first two hours served as an acclimatization period while the last hour was the actual recorded response to the drug. Since the animals were awake and quiet, but not fasting, the quantity of oxygen consumed should be regarded as the resting, not the basal, metabolism.

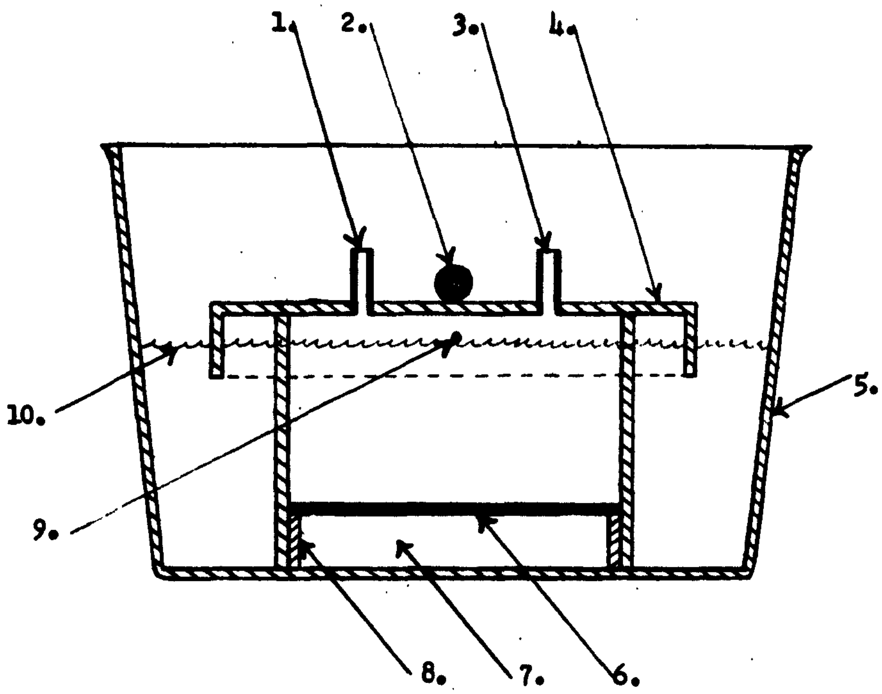
To control the environmental temperature, the chamber was immersed in a thermostatically controlled water bath (Forma Temperature Bath, model #2075, Forma Scientific, Inc., Marietta, Ohio).

A typical oxygen consumption experiment is summarized as follows: During an individual experiment the animal was placed in a plastic chamber (Fig. 1). The chamber was large enough to permit the animal freedom of movement. A wire screen separated the animal from the bottom well of the chamber, which was covered with soda lime for carbon dioxide absorption. The chamber was immersed (with animal) in the water bath; pure oxygen (100%) was used to flush the apparatus. The apparatus was flushed once; the instrument was then recharged with pure oxygen and the experiment began. At the end of the two-hour acclimatization period (which served as a baseline) the chamber was removed from the temperature bath. At this point, either saline (if it were a control animal) or a known concentration of the drug Compazine was injected intraperitoneally. The animal chamber was replaced in the temperature bath, flushed with pure oxygen and then recharged. This last hour was the actual recorded response to the drug. It was not feasible in this experiment to obtain body temperature as metabolic measurements were conducted.

The drug Compazine was obtained from the Smith, Kline and French Laboratories Research and Development Division,

FIGURE 1

Plastic chamber with the following components: 1. outlet to recording spirometer; 2. clamping rod; 3. inlet from oxygen source; 4. lid; 5. chamber; 6. perforated shelf; 7. soda lime absorbent; 8. insert; 9. rubber stopper; and 10. water level.


1.
2.
3.
4.
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7.
8.
9.
10.

Philadelphia, Pa.; (SK & F, #4657-J); 28-Ped; U. S. Pat. No. 2,902,484; Iso #126195). I am indebted to this company for the generous supply of the drug which was made available.

Eight different concentrations of drug in mg./kg. body weight were used in this experiment: (10 mg./kg., 20 mg./kg., 50-100 mg./kg.)(10 mg./kg. gradations). Each concentration of drug was employed at both temperatures, namely, 18° and 26° C. The particular drug concentration which was to be used on a particular day was freshly prepared. The drug was readily soluble in distilled water at room temperature.

At 26° C., six animals (randomly selected) were used for controls and for each drug concentration. At 18° C., only four animals were used for controls and for each drug concentration. The limited number of gerbils available for experimentation dictated that only four animals be used. Some of the animals received more than one drug dosage and the possibility of building up a cummulative action of the drug had to be considered. As a result, at least ten days (usually 2-3 weeks) elapsed before the same animal was used again. In this experiment, the "letter and spirit of the principles," as outlined in Appendix E, were followed in the care and use of animals.

In addition to recording the oxygen consumed for each animal for three hours, 24 hours after the animal had its baseline determined, oxygen consumption was again measured for one hour on the animals--beginning with the drug dosage of 60 mg./kg. and continuing through 100 mg./kg. Those animals which received drug dosages of 90 mg./kg. and 100 mg./kg. had oxygen consumption values determined 48 hours after the initial injection, in addition to the previously mentioned determinations. The reason for running these oxygen consumption checks 24 and 48 hours after the initial injection was to ascertain how fast the drug was being detoxified. Oxygen consumption was recorded in ml. O_2/gm./hr. which was corrected to standard conditions of temperature and pressure. The mean of each group was used in the plotting of all graphs.

The results which were obtained were subjected to the following statistical analysis: an analysis of variance, involving the two criteria of classification, more than one entry in each box method, was determined (Croxton, 1953) (Appendix F).

Any variations, from the above description, in the number of animals used at a particular concentration or temperature will be pointed out later at an appropriate place in table or text.

RESULTS

The results of the experiments on oxygen consumption are summarized in Table I. It is evident by inspection that at the lower environmental temperature (18° C.), oxygen consumption values are higher than at the 26° C. temperature. Statistical analysis confirms this observation (Appendix F). As drug dosage increases at 18° C., it is apparent that there is a slight fall in oxygen consumption up to the 60 mg./kg. dose. At this dosage (60 mg./kg.) and above (70 mg./kg. and 80 mg./kg. doses respectively), there is an increase in oxygen consumption above the control value (Figure 2); oxygen consumption rapidly declines beginning at the 90 mg./kg. dose.

At 26° C. the initial drop in oxygen consumption (with doses of 10, 20, and 50 mg./kg. respectively) is more pronounced than at 18° C. Again, a marked increase in oxygen consumption was observed at the 60 mg./kg. dose; a gradual decline of oxygen uptake began at the 90 mg./kg. dose. Oxygen consumption did not rise above control values at the 26° C. temperature (Figure 3) regardless of the particular dose used.

Only three animals were used for each temperature at the dose of 100 mg./kg., because at the 26° C. temperature this dosage killed the animals approximately 60 hours after the injection. All three animals at 18° C. survived with no apparent ill-effects.

The oxygen consumption values, 24 and 48 hours after the baseline determinations, are summarized in Table II. In general, the oxygen consumption values at 18° C., twenty-four hours after the injection, show an increase above baseline values; the forty-eight hour values for oxygen consumption after dosages of 90 mg./kg. and 100 mg./kg. respectively, show an increase above the 24 hour value at this temperature. This situation, an increase in oxygen consumption after 24 hours, is not true for a drug dosage of 100 mg./kg. at 26° C., although it is true for a dose of 90 mg./kg. In general, at 26° C. the 24 hour values for oxygen consumption showed an increase above the baseline value except for the drug dosage of 100 mg./kg. Both 48 hour values for drug dosages of 90 mg./kg. and 100 mg./kg. showed slight increases above the 24 hour value. Graphic representations of these oxygen consumption values are shown in Figures 2 and 3.

A more effective comparison of results obtained at these two temperatures (excluding the 24 and 48 hour values) with regard to drug dosage is shown in Figure 4. Here, oxygen consumption, at each concentration, is compared to the per cent of control values at each temperature.

The measurement of oxygen consumption (after the injection of Compazine) was begun approximately five minutes after the animal had been placed in the constant temperature bath. The delay was a result of flushing and recharging the instrument. It was observed that, shortly after the drug was administered, the animal became lethargic and the hind limbs were spread out. Therefore, no further wait was deemed necessary between the injection and actual recording of oxygen consumption. When the 24 and 48 hour values for oxygen consumption were ascertained, the temperature bath was brought to the desired temperature; the animal was placed in the bath and again approximately five minutes elapsed before oxygen consumption was started. There was no acclimatization period before recording the 24 and 48 hour values for oxygen consumption.

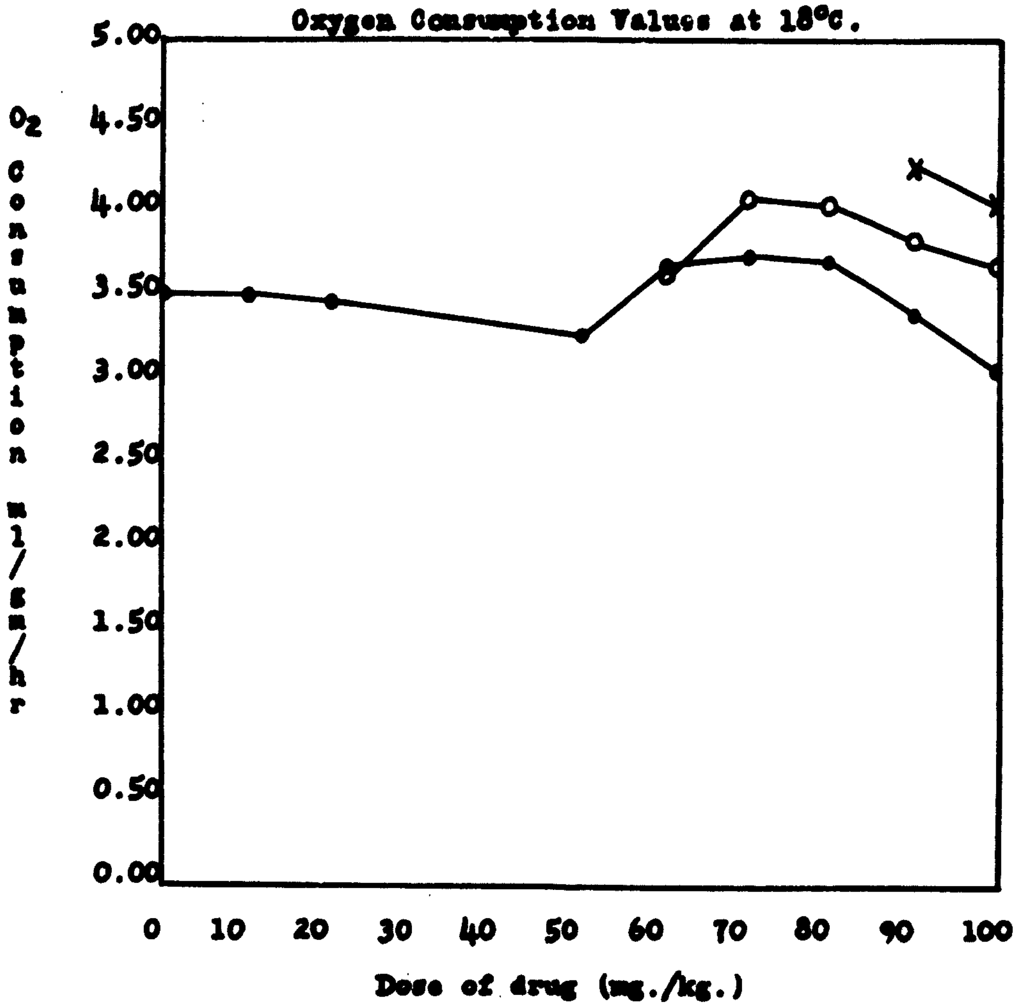
FIGURE 2

Graph showing oxygen consumption values at 18°C.

●-----● = oxygen consumption values after initial injection of drug;

o-----o = oxygen consumption values 24 hours after the initial injection; and

x-----x = oxygen consumption values 48 hours after the initial injection.



Oxygen Consumption Values at 18°C.
O_2 Consumption ml/gm/hr
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0
10
20
30
40
50
60
70
80
90
100
Dose of drug (mg./kg.)

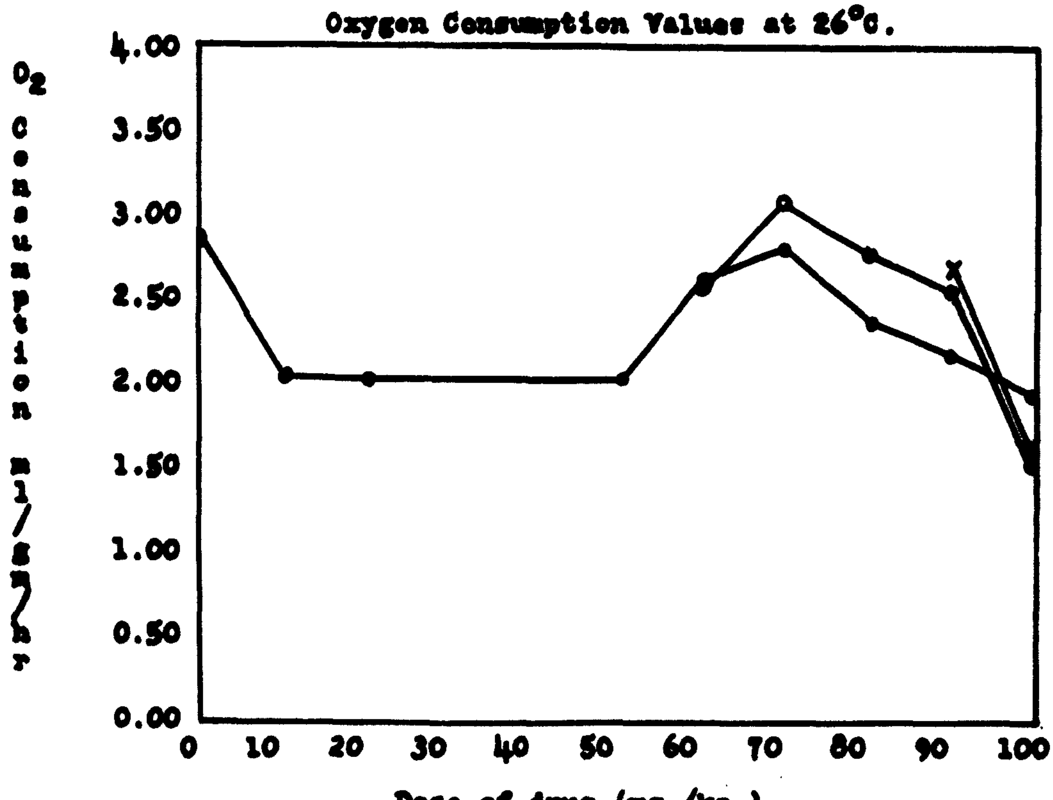
FIGURE 3

Graph showing oxygen consumption values at 26°C.

●-----● = oxygen consumption values after initial injection of drug;

o-----o = oxygen consumption values 24 hours after the initial injection; and

x-----x = oxygen consumption values 48 hours after the initial injection.



Oxygen Consumption Values at 26°C.
O_2 Consumption ml/gm/hr
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0
10
20
30
40
50
60
70
80
90
100
Dose of drug (mg./kg.)

FIGURE 4

Graph showing oxygen consumption in relation to the per cent of control values. Both the 18°C. and 26°C. values are after the initial injection of the drug.

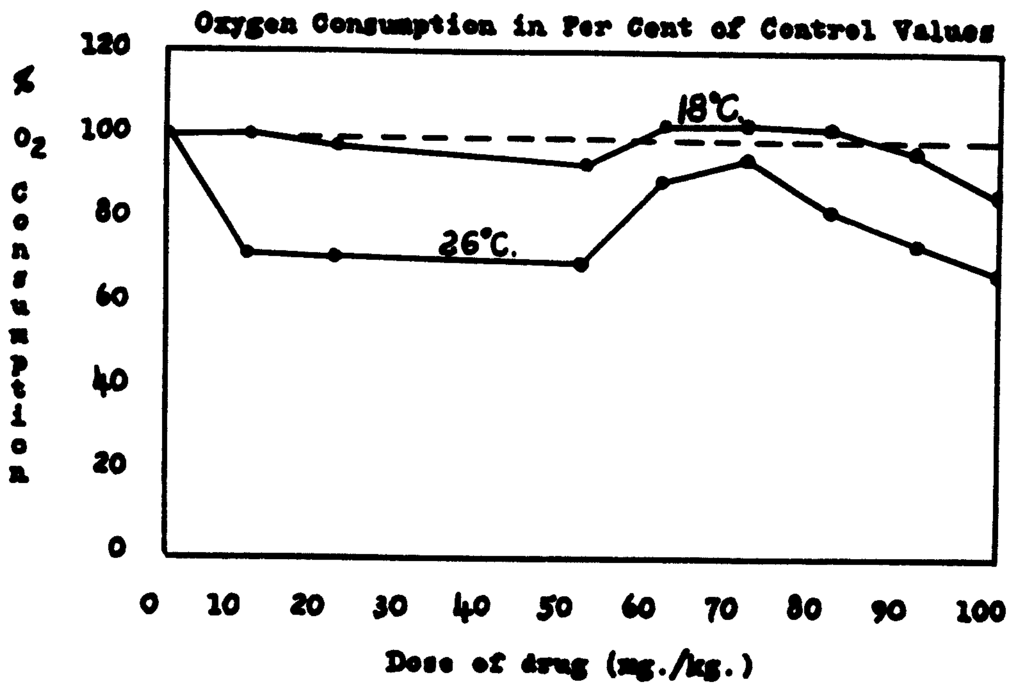

Oxygen Consumption in Per Cent of Control Values
% O_2 Consumption
120
100
80
60
40
20
0
18°C.
26°C.
0
10
20
30
40
50
60
70
80
90
100
Dose of drug (mg./kg.)

TABLE I

OXYGEN CONSUMPTION VALUES AFTER INITIAL INJECTION OF DRUG AT 18°C. AND 26°C.

Dose of Drug (mg./kg.)	Total Animals Used	Avg. Wt. (gms.)	Oxygen Consumed (ml O_2/gm/hr)
18°C.			
Controls	5	55.0	3.49 ± .44
10	4	84.0	3.50 ± .47
20	4	77.5	3.46 ± .35
50	4	78.7	3.26 ± .34
60	4	73.7	3.68 ± .32
70	4	72.0	3.72 ± .48
80	4	63.0	3.71 ± .31
90	4	78.0	3.42 ± .11
100	3	79.7	3.06 ± .19
26°C.			
Controls	6	59.6	2.88 ± .56
10	6	71.1	2.06 ± .31
20	6	72.0	2.04 ± .43
50	6	85.3	2.01 ± .43
60	6	64.8	2.60 ± .34
70	6	69.8	2.79 ± .58
80	6	74.3	2.36 ± .61
90	6	77.3	2.17 ± .44
100	3	74.6	1.94 ± .28

TABLE II

I = Oxygen consumption after initial injection of drug
II = Oxygen consumption 24 hours after the initial injection of drug
III = Oxygen consumption 48 hours after the initial injection of drug I, II, III = (ml. O_2/gm./hr.)

18°C.

Dose of Drug (mg./kg.)	Number of Animals	I	Number of Animals	II	Number of Animals	III
60	4	3.68	4	3.62	---	---
70	4	3.72	4	4.07	---	---
80	4	3.71	4	4.02	---	---
90	4	3.42	4	3.84	4	4.27
100	3	3.06	3	3.70	3	4.02

26°C.

Dose of Drug (mg./kg.)	Number of Animals	I	Number of Animals	II	Number of Animals	III
60	6	2.60	5*	2.58	---	---
70	6	2.79	6	3.05	---	---
80	6	2.36	6	2.77	---	---
90	6	2.17	6	2.55	5*	2.64
100	3	1.94	1*	1.51	1*	1.58

* Technical difficulties prevented utilizing the full number of animals

DISCUSSION

Temperature Expressions

Adolph (1951) holds that, "diverse animals' responses to temperature changes have been most frequently compared on the basis of their oxygen consumptions." Studies of oxygen consumption in animals indicate that there is an increase in oxygen consumption with a decrease in ambient temperature (Benedict and Petrick, 1930; Dawson, 1955; Herrington, 1940; Pearson, O.P., 1960; Robinson and Henrickson, 1961; Robinson, 1959; and Sullivan and Mullen, 1954). This increase in oxygen consumption is especially marked below the critical temperature. The term "critical temperature" is used to define the lowest ambient metabolic condition. However, if one is expressing oxygen consumption in terms of colonic temperature or surface temperature, there is usually a decrease in oxygen consumption with decreased temperature.

Oxygen consumption in rats usually decreases linearly with colonic temperature (Adolph, 1950). However, there is a temperature range in which oxygen consumption increases with decreasing colonic temperature. Adolph (1950) has shown in the rat that a decrease in colonic temperature from 37°C. to 35°C. is accompanied by a sharp rise in oxygen consumption, resulting presumably from shivering. Below 35°C. there is a linear decrease in oxygen consumption until lethal temperature, which, for the rat, is approximately 15°C. If oxygen consumption is measured in reference to skin temperature, oxygen consumption increases from 37°C. to 32°C. and then begins to decrease linearly until lethal temperature (Adolph, 1950).

However, the studies by Blood et. al. (1949) in rats indicates that "although a reduced consumption of oxygen is in general accompanied by a fall in body temperature, instances occurred where the two factors apparently varied independently of each other." They accounted for this variance by noting that "heat production may not depend solely on oxygen comsumption, since anaerobic, exothermic reactions may contribute, and whether variations in heat production will affect body temperature will depend on the rate of heat loss."

Thus, it is clearly important to specify what temperature (i.e., colonic, skin, ambient) is the reference when attempting a correlation of temperature with metabolism.

The present study indicated a rise in oxygen consumption with a decrease in ambient temperature and the fact that the ambient temperature was below the thermal neutrality range (30° - 40°C.) of this species (Robinson, 1959) would lend support to this statement. It was not feasible to measure rectal temperature and oxygen consumption simultaneously. No comment on rectal temperature is possible.

Table III shows a comparison of oxygen consumption in selected desert mammals at temperatures 18°and 26°C. From the table it can be seen that the range of oxygen consumption is fairly constant at the 26°C. temperature with a slightly larger variation occurring at the 18°C. temperature among these desert mammals. It becomes evident that the general metabolic patterns of desert mammals are thus closely related.

Effect of Temperature on Drug Action

Small animals are often used to study the effects of temperature and the action of drugs. It is therefore important to know the factors which may influence the results. It has been recognized for many years that the environmental temperature may modify the toxicity of a chemical by changing body temperature (Fuhrman and Fuhrman, 1961). However, toxicity and duration or intensity of action may be affected differently by changes in body temperature. The duration or intensity of action may in turn be affected by absorption and detoxification of the drug. Shemano and Nickerson (1958) have shown that each of the drugs that they studied had a critical ambient temperature; i.e., a temperature above which hyperthermia occurred and below which hypothermia resulted. They hold that the effect of ambient temperature on thermal response to drugs interferes with the central body temperature regulator. Shemano and Nickerson (1958) state: "It is only within the central nervous system that

TABLE III

A COMPARISON OF OXYGEN CONSUMPTION IN DESERT MAMMALS

Animal	Avg. Wt. (gms.)	Total Animals Used	Temp. °C.	Oxygen Consumed (ml O_2/gm/hr)	Temp. °C.	Oxygen Consumed (ml O_2/gm/hr)	Reference
Meriones unguiculatus	55.0 59.6	5-18°C. 6-26°C.	18	3.49	26	2.88	Present Work
Meriones unguiculatus	61-80	9	18	3.12	26	2.00	Robinson (1959)
Gerbillus pyramidum	72-145	21	18	2.05	26	1.23	Robinson and Henrickson (1961)
Dipodomys merriami	34.7	25	18	3.98	26	2.54	Dawson (1955)
Dipodomys panamintinus	56.9	15	18	3.72	26	2.46	Dawson (1955)
Citellus leucurus	79.2	12	18	4.06	26	2.45	Dawson (1955)

the various factors involved in temperature regulation are integrated, and it is in this area that defenses against both hyperthermia and hypothermia can be impaired by a single drug action."

The present studies indicate that there is a definite interaction between drug concentration and temperature at the temperatures chosen (Fig. 4). The pattern of response (oxygen consumption) to the drug is similar at both temperatures (18° and 26°C.). However, oxygen consumption values are higher at 18°C. than they are at 26°C. In comparing figures 2 and 3, it will be seen that the oxygen consumption value (48 hour), at a dose of 100 mg./kg. and at 18°C., is higher than the value at the same dose immediately after injection. At 26°C. the oxygen consumption value (48 hour), at a dosage of 100 mg.kg., is lower than the value at the same dose immediately after injection. It was previously stated that the gerbils receiving a dose of 100 mg./kg., at 26°C., died approximately 60 hours after the initial injection while those gerbils receiving the same dose at 18°C. all survived. The fact that all the animals died at this concentration (100 mg./kg.) and temperature (26°C.) while those at 18°C. survived would suggest that at the lower ambient temperature the drug was less toxic.

Shemano and Nickerson (1958) observed that a dose of 22 mg./kg. of Thorazine, injected subcutaneously into rats, produced, within one hour, hyperthermia at 39°C. (ambient) and hypothermia at 30°C. (ambient). Colonic temperature rose from approximately 40°C. to 41.5°C. at the 39°C. ambient temperature whereas colonic temperature fell from approximately 37°C. to 35°C. at the 30°C. ambient temperature. They also showed that Chlorpromazine does not depress basal metabolism but rather produced cutaneous vasodilation; it appears probable that enhanced heat loss is a major component of the hypothermic effect produced by Chlorpromazine. The fact that Compazine, as well as Thorazine, is a phenothiazine and a major tranquilizer suggests that the generalized actions of the two agents may be similar.

In attempting to explain the rise in oxygen consumption which occurred at the drug dosages of 60-70 mg./kg. (Fig. 4), at both temperatures, the following possibility is suggested: The drug showed a depressant effect on oxygen consumption for the drug doses 10, 20, and 50 mg./kg. at the 26°C. temperature. However, there is less of a depressant effect at 18°C. than at 26°C. with these doses. The fact that 18°C. is further below thermal neutrality (30°-40°C.), than is 26°C., might account for the increased values (oxygen consumption) at 18°C. At doses of 60 and 70 mg./kg. there is a pronounced rise in oxygen consumption at both temperatures. It will be seen from figure 4 that, at the 26°C. temperature, the oxygen consumption values did not rise above the control values, but that at 18°C. the oxygen consumption values actually rose above control values. The fact that at 18°C. the oxygen consumption values rose above control values suggests, that at this temperature, there is a synergistic effect between drug and temperature. At 26°C. there is less of a synergistic effect between drug and temperature since this temperature is only slightly below thermal neutrality. At higher doses there is a beginning decline in oxygen consumption and this is probably due to the increasing toxicity of the drug.

Effect of Using Pure Oxygen

Previous work on the effect of using pure oxygen in metabolic studies has been inconclusive. Early workers in this field reported that breathing pure oxygen did not alter any of the vital processes, i.e., respiration and circulation were neither accelerated nor retarded and temperature remained unchanged (Bean, 1945). Later workers were not all in accord with these views; at the present time the effects produced by using hyperoxygenated air are still in doubt. It must be remembered that a large effect is not to be expected, because the oxygen supply to the tissues will only be increased by the extra amount of oxygen physically dissolved in the arterial blood which cannot be more than 10 per cent of the total quantity carried (Ohlsson, 1947).

In this experiment the control values of oxygen consumption are higher at both temperatures than those reported by Robinson (1959) on the same species (Table III). This difference is much more marked at 26°C. than at 18°C. This difference at 26°C. is believed to result largely from the fact that 100 per cent oxygen was used; Robinson simply used air. Other factors which might account for this difference would be age, sex, diet, etc., but none of these factors are more convincing than the one suggested.

Bean (1945) points out that breathing hyperoxygenated air does cause alterations in pulse rate. "This change in cardiac activity in itself, clearly indicates that breathing increased percentages of oxygen alters the physiological activity of tissues, and in final analyses such alterations must involve changes in fundamental metabolic processes, even though no coincident exchange in gaseous interchange may be readily detected by the techniques usually employed."

Expression of Standard Metabolic Rates

Metabolic rates are often expressed as the basal metabolic rate, a condition which is applicable only to human beings, since it is difficult to determine when an animal is in a post-absorptive state. Amberson and Smith (1948) define basal metabolism as a condition which is "just sufficient to keep the resting, quietly breathing, body alive."

Davson (1960) notes that "the term 'basal metabolism', applying as it does to the heat production of the resting homoiotherm at its thermal neutrality, cannot very well be applied to the poikilotherm, hence one speaks, instead, of the standard metabolism, measured under conditions approximating as closely as possible to those laid down for measurements on homoiotherms."

In this experiment the animals were awake and quiet, but not fasting, and therefore the expression of total oxygen consumption should be regarded as the resting, not the basal metabolism. Herrington (1940) points out that since no measurements under any condition are ever strictly basal, there appear to be no serious objections to accepting "normalized" activity by populations and long periods as a condition of the standard metabolism. This is in accord with Harvey (1958) who states: "As this condition is difficult if not impossible to obtain and to assess in small animals it is better to aim at achieving a less absolute value." The exact reason for desiring basal values is not understandable when the conditions under basal metabolism are somewhat different from those under which animals normally live as Kleiber (1947) has pointed out.

Metabolic rate may be expressed as oxygen used or heat produced per unit of various functions of body size. Kleiber and Cole (1950) have noted the following expressions of metabolic rate: (a) the metabolic rate per unit weight offers an overall measure for the intensity of tissue metabolism; (b) the metabolic rate per unit surface or of the 2/3 power of body weight is important for questions of heat exchange; and (c) the metabolic rate per unit of the 3/4 power of body weight on the average eliminates best the effect of size in interspecific comparison. Table IV gives the oxygen consumption and metabolic rates of various animals with the above metabolic expressions. Kleiber, Smith, and Chernikoff (1956) note that none of the various units of body size in which metabolic rate may be expressed is absolutely superior to all others.

In fact there may be some question as to the reliability of some of these expressions. Rodbard (1950) says that "studies on the metabolic rates of animals have been based on a calculated surface area based upon approximately the 0.7 power of body weight. Since the weight of the animal appears to affect the body temperature, which in itself plays a significant role in metabolic rate of the animal, simple conversion of weight to surface area may lead to considerable error. This is apparent upon consideration of the fact that a variation of only 1°C. in body temperature may increase the resting metabolism by 10 per cent. It would therefore appear that comparisons of the metabolic activity of various species on the basis of surface area alone, without

TABLE IV

OXYGEN CONSUMPTION AND METABOLIC RATE OF VARIOUS MAMMALS

Rodent	Temp °C.	Total Animals Used	Avg. Wt. (gms.)	Oxygen Consumed ($ml O_2$/gm/hr)	Oxygen Consumed ($ml O_2$/$gm^{2/3}$/hr)	Oxygen Consumed ($ml O_2$/$gm^{3/4}$/hr)	Cal./24hr	Reference
Meriones unguiculatus	26	5	55.0	3.49	1.33	1.73	22.1	Present Work
Meriones unguiculatus	26	9	61-80	3.12	1.23-1.35	1.53-1.70	21.8-28.7	Robinson (1959)
Gerbillus pyramidum	26	21	72-145	2.05	.85-1.08	1.07-1.26	17.0-34.2	Robinson & Henrickson (1961)
Citellus leucurus	26	12	79.2	4.06	1.74	2.16	37.0	Dawson (1955)
Cavia porcellus	25	1	82.0	1.66	.72	.89	15.7	Sullivan & Mullen (1954)
Mesocricetus auratus	25	1	751	0.35	.31	.34	30.2	Sullivan & Mullen (1954)
Citellus barrowensis	25	1	620	0.67	.57	.59	47.8	Sullivan & Mullen (1954)

regard to body temperature, are likely to be misleading. It is noteworthy that closely related species may have fairly deviant body temperature in accordance with their body weights."

Conrad and Miller (1956) point out that the oxygen consumption at rest and at its "critical" environmental temperature increases with age, but the relative rates of the increase of the two are different. They explain this by assuming that the oxygen consumption depends upon the "activity" and amount of metabolically active tissue while the actual body weight depends upon the amount of metabolically active tissue plus inert material (e.g., fat and water). They point out that it is conceivable that during different stages of development the "activity" and the rate of production of metabolic material are different, the rate of production of inert material may also vary. Therefore, these considerations make it illogical to expect a constant relationship between oxygen consumption and body weight, or any power of body weight, throughout the life of an animal."

CONCLUSIONS

1. Oxygen consumption increases with a decrease in ambient temperature. At 18°C. the control value for oxygen consumption was 3.49 ml. O_2/gm./hr.; at 26°C. the control value was 2.88 ml. O_2/gm./hr.
2. Compazine depresses, at drug doses of 20 and 50 mg./kg., oxygen consumption at 18°C. Compazine shows a depressant effect for 10, 20 and 50 mg./kg. at the 26°C. temperature. There is less of a depressant effect at 18°C.
3. There is a pronounced rise in oxygen consumption at a drug dose of 60 mg./kg. at both temperatures.
4. Compazine, in doses of 90-100 mg./kg., begins to show toxicity effects, especially at the 26°C. temperature.
5. Statistical analysis (analysis of variance) reveals a definite interaction between drug concentration and temperature.
6. Small desert rodents appear to have closely related metabolic rates as far as oxygen consumption is concerned.

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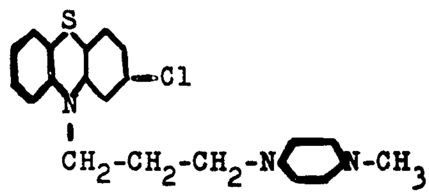
APPENDICES

APPENDIX A

Chemistry

Formula: 'Compazine': $C_{20}H_{24}N_3ClS = 373.96$



Generic name - prochlorperazine

Chemical name - 2-Chloro-10-(3-(1-methyl-4-piperazinyl)-propyl)-phenothiazine.

In 'Compazine' tablets, the compound is present as the dimaleate salt (2 HOOC-CH-CH-COOH). In 'Compazine' ampuls, the compound is present as the ethane disulfonate salt. ($HO_3S\text{-}CH_2\text{-}CH_2\text{-}SO_3H$).

Composition

Prochlorperazine dimaleate:

Mol. wt. = 606.11
% Base = 61.7
% Maleic acid = 38.3

Prochlorperazine ethane disulfonate:

Mol. wt. = 564.16
% Base = 66.3
% Ethane disulfonate = 33.7

APPENDIX B

CLASSIFICATION AND CHARACTERIZATION OF THE TRANQUILIZERS

MAJOR TRANQUILIZERS	MINOR TRANQUILIZERS
(a) Phenothiazine Group	(a) Diol-carbamate Group
(b) Rauwolfia Group	(b) Diphenylmethane Group
	(c) Miscellaneous Group

THE MAJOR TRANQUILIZERS ARE CHARACTERIZED BY THE FOLLOWING POINTS:

1. These drugs produce a type of emotional calmness with relatively little sedation; they have proved useful in controlling the symptoms of acutely and chronically disturbed psychotic patients.
2. They are capable of producing the reversible extra-pyramidal syndrome characterized by rigidity, tremors, and drooling.
3. The incidence of annoying side reactions is relatively high with the use of these drugs, and serious dangers do exist to some extent.
4. They produce little, if any dependency or habituation.

THE MINOR TRANQUILIZERS ARE CHARACTERIZED BY OTHER POINTS:

1. These drugs produce a type of calmness or relaxation, but not of the same quality as that produced by the major tranquilizers. They may have a mild sedative effect but are not highly effective in treating disturbed

psychotic patients. They are useful in the treatment of psychoneurotic problems and common nervous tension.

2. They do not produce the extrapyramidal motor phenomena so characteristic of the major tranquilizers.
3. The incidence of annoying side reactions with the use of these drugs is relatively low. Dangerous reactions are rare.
4. Habituation may occur with the use of some of these agents.

Literature Cited:

Benson, W. M. and Schiele, B. C. 1962. Tranquilizing and Antidepressive Drugs. Charles C. Thomas, Springfield, 89p.

APPENDIX C

TABLE 1

PHARMACOLOGIC EFFECTS OF TRANQUILIZERS

Type of Action	Majors: Phenothiazines (chlorpromazine)	Majors: Rauwolfias (reserpine)	Minors: Diphenylmethanes (benactyzine)	Minors: Diol-carbamates (meprobamate)
Response to afferent stimulation	-	-	-	-
Posterior hypothalamic activity	-	-	-	-
Conditioned reflexes	-	-	-	0
Blood pressure	-	-	0	0
Body temperature	-	-	0	0
Pupillary size	0	-	0	0
Effect on Muscle Tension	0	0	0	-
Intestinal activity	0	+	-	0
Antihistaminic activity	+	0	+	0
Adrenergic antagonism	+	+	0	0
Occurrence of tremors	+	+	0	0
Tendency for seizures	+	+	+	-
Rhinencephalic system activity	+	+	U	-

(0) No significant effect (+) Increased (U) Unknown (-) Decreased

Literature Cited: Benson, W. M. and Schiele, B. C. 1962. Tranquilizing and Antidepressive Drugs. Charles C. Thomas, Springfield, 89p.

APPENDIX D

GENERAL DESCRIPTION OF THE RESPIROMETER

This spirometer is a sensitive instrument capable of measuring extremely small volume changes in a closed system. It accomplishes this with virtually zero pressure loading of the system. The volume discrimination of the instrument is 1/4 cc or less and the pressure variations experienced are of the order of a fraction of a millimeter of water.

The principle of operation is best described by assuming the inlet tube of the spirometer connected to a syringe. A closed system is then defined, and the spirometer servo-mechanism continually maintains zero pressure in that system. If the syringe plunger were pulled out slightly, a small increase in total system volume would result. However, a very sensitive volume transducer in the instrument detects this increase and generates a command that the motor move the piston to such a position as to nullify this volume increase. Thus the piston will follow any volume changes introduced from the syringe.

A recording pen, connected rigidly to the rack which moves the piston, records the exact motion of the piston on a strip chart. The time base is provided by means of a synchronous motor rotating a cam. Once every minute the pen rod support drops approximately 2 mm, so that the pen is deflected momentarily 2 mm downward. Thus the volume change occurring in any one minute interval during a run can be evaluated by oberving the distance between two successive time markers.

The piston moves in an accurate cylinder, so linear travel can be related properly to volume change. The cross section of this cylinder is 6.73 cm^2, and the accurate volume calibration figure is 0.673 cubic centimeters per millimeter of pen travel. However, for convenience a calibration figure of 2/3 cc per mm travel may be used with less than 1 per cent error.

APPENDIX E

GUIDING PRINCIPLES IN THE CARE AND USE OF ANIMALS

(Approved by the Council of the American Physiological Society)

Only animals that are lawfully acquired shall be used in this laboratory, and their retention and use shall be in every case in strict compliance with state and local laws and regulations.

Animals in the laboratory must receive every consideration for their bodily comfort; they must be kindly treated, properly fed, and their surroundings kept in a sanitary condition.

Appropriate anesthetics must be used to eliminate sensibility to pain during operative procedures. Where recovery from anesthesia is necessary during the study, acceptable technic to minimize pain must be followed. Curarizing agents are not anesthetics. Where the study does not require recovery from anesthesia, the animal must be killed in a humane manner at the conclusion of the observations.

The postoperative care of animals shall be such as to minimize discomfort and pain, and in any case shall be equivalent to accepted practices in schools of Veterinary Medicine.

When animals are used by students for their education or the advancement of science such work shall be under the direct supervision of an experienced teacher or investigator. The rules for the care of such animals must be the same as for animals used for research.

Charles H. Wilbe

Director of Laboratory

APPENDIX F

Computation of Values Required for Analysis of Variance of Data between Drug Concentration and Temperature

Observed Data and Sums for Columns and Rows

Dose (mg./kg.)	18°C. (ml O_2/ gm/hr)	26°C. (ml O_2/ gm/hr)	$\sum_{1}^{N_r} X$	Dose	18°C.	26°C.	$\sum_{1}^{N_r} X$
Controls	2.83 3.30 3.70 4.03 3.93	3.70 4.06 3.05 3.41 2.47 4.00	38.48	70	4.37 4.28 3.84 3.16	3.87 2.97 4.06 3.19 2.37 3.69	35.80
10	3.25 4.56 3.92 4.11	3.32 2.52 2.65 3.17 2.60 2.67	32.77	80	4.55 3.73 4.06 4.43	3.64 3.60 3.29 3.95 2.70 4.65	38.59
20	5.04 4.99 5.29 4.34	3.36 2.62 3.09 3.73 3.04 2.46	37.96	90	4.26 4.30 4.01 4.21	3.64 2.81 2.89 3.84 3.09 3.88	36.93
50	4.08 3.82 4.55 4.66	3.47 2.57 2.20 2.95 2.29 2.89	33.48	100	3.61 4.08	3.35 3.56	21.40
60	3.56 3.97 3.41 4.20	3.60 3.18 3.24 2.64 2.73 3.39	33.92	$\sum_{1}^{N_c} X$	146.32	163.01	309.33=$\sum X$

Squares and Sums for Columns and Rows

Dose (mg./kg.)	18°C. (ml O_2/ gm/hr)	26°C. (ml C_2/ gm/hr)	$\sum_{1}^{N_r} X^2$
Controls	8.00 10.89 13.69 16.24 15.44	13.69 16.48 9.30 11.62 6.10 16.00	137.45
10	10.56 20.79 15.36 16.89	11.02 6.35 7.02 10.04 6.76 7.12	111.91
20	25.40 24.90 27.98 18.83	11.28 6.86 9.54 13.91 9.24 6.05	153.99
50	16.64 14.59 20.70 21.71	12.04 6.60 4.84 8.70 5.24 8.35	119.41
60	12.67 15.76 11.62 17.64	12.96 10.11 10.49 6.96 7.45 11.49	117.15
70	19.09 18.31 14.74 9.98	14.97 8.82 16.48 10.17 5.61 13.61	131.78
80	20.70 13.91 16.48 19.62	13.24 12.96 10.82 15.60 7.29 21.62	152.24
90	18.14 18.49 16.08 17.72	13.27 7.89 8.35 14.74 9.54 15.05	139.24
100	13.03 16.64 15.21	11.22 12.67 8.41	77.18
$\sum_{1}^{N_c} X^2$			$1140.35=\sum X^2$

Sums and Squares of Sums for Boxes

Box	$\sum_{1}^{N_b} X$	$\left(\sum_{1}^{N_b} X\right)^2$
Row 1, Col. 1	17.79	316.48
Col. 2	20.69	428.07
Row 2, Col. 1	15.84	250.90
Col. 2	16.93	286.62
Row 3, Col. 1	19.66	386.51
Col. 2	18.30	334.89
Row 4, Col. 1	17.11	292.75
Col. 2	16.37	267.97
Row 5, Col. 1	15.14	229.21
Col. 2	18.78	352.68
Row 6, Col. 1	15.65	244.92
Col. 2	20.15	406.02
Row 7, Col. 1	16.76	280.89
Col. 2	21.83	476.54
Row 8, Col. 1	16.78	281.56
Col. 2	20.15	406.02
Row 9, Col. 1	11.59	134.32
Col. 2	9.81	96.23
TOTAL	309.33	5472.58

ANALYSIS OF VARIANCE COMPUTATION

(Two criteria of classification, more than one entry in each box)

To enable the necessary tests, several symbols must be clarified. $\bar{X}_r$ = the mean of a row; N_r = the number of items in a row; k_r = the number of rows; $\sum_1^{N_r}$ = a sum over the N_r items in a row; and $\sum_1^{k_r}$ = a sum over the k_r rows. In the two criteria of classification test there are possible five sources of variation; total, between column means (between temperatures), between row means (drug concentration), interaction between columns and rows, and within boxes.

$$(\sum X)^2 = (309.33)^2 = \underline{95,685.05}$$

$$\sum_1^{k_c}\left(\sum_1^{N_c} X\right)^2 = (146.32)^2 + (163.01)^2 = 21409.54 + 26572.26 = \underline{47981.80}$$

$$\sum_1^{k_r}\left(\sum_1^{N_r} X\right)^2 = (38.48)^2 + (32.77)^2 + (37.96)^2 + (33.48)^2 + (33.92)^2 + (35.80)^2 + (38.59)^2 + (36.93)^2 + (21.40)^2 = \underline{10859.34}$$

TOTAL VARIATION:

$$\sum X^2 - \frac{(\sum X^2)}{N} = 1140.35 - \frac{(309.33)^2}{87} =$$

$$1140.35 - 1099.83 = \underline{40.52}$$

VARIATION BETWEEN COLUMN MEANS:

$$\frac{\sum_{1}^{k_c}\left(\sum_{1}^{N_c} X\right)}{N_c} - \frac{(\sum X)^2}{N} = \left(\frac{21409.54}{36} + \frac{2[illegible]72.26}{51}\right) - \frac{95685.05}{87}$$

$$= 1115.72 - 1099.83$$

$$= \underline{15.89}$$

VARIATION BETWEEN ROW MEANS:

$$\frac{\sum_{1}^{k_r}\left(\sum_{1}^{N_r} X\right)}{N_r} - \frac{(\sum X)^2}{N} = \left(\frac{1480.71}{11} + \frac{1073.87}{10} + \frac{1440.96}{10} + \right.$$

$$\frac{1120.91}{10} + \frac{1150.56}{10} + \frac{1781.64}{10} +$$

$$\left.\frac{1489.18}{10} + \frac{1363.82}{10} + \frac{457.96}{6}\right) -$$

$$\frac{95685.05}{87}$$

$$= 1102.27 - 1099.83$$

$$= \underline{2.44}$$

VARIATION WITHIN BOXES:

$$\sum x^2 - \frac{\sum_1^{k_b}\left(\sum_1^{N_b} x\right)^2}{N_b} = 1140.35 - \left(\frac{316.8}{5} + \frac{428.4}{6} + \frac{249.6}{4} + \frac{285.6}{6} + \frac{384.1}{4} + \frac{334.9}{6} + \frac{292.4}{4} + \frac{268.9}{6} + \frac{228.0}{4} + \frac{353.4}{6} + \frac{243.3}{4} + \frac{404.0}{6} + \frac{282.2}{4} + \frac{475.2}{6} + \frac{282.2}{4} + \frac{404.0}{6} + \frac{134.5}{3} + \frac{96.04}{3}\right)$$

$$= 1140.35 - 1122.00$$

$$= \underline{18.35}$$

INTERACTION:

$$\sum_1^{k_b} \left[N_b(\bar{X}_b + \bar{X} - \bar{X}_r - \bar{X}_c)^2\right]$$

$$40.52 - (15.89 + 2.44 + 18.35) = \underline{3.84}$$

Summary of Computations for Analysis of Variance

Source of Variation	Amount of Variation	Degrees of Freedom	Estimated Variance
Between column means	15.89	1	15.89
Between row means	2.44	8	.305
Interaction	3.84	8	.48
Within Boxes	18.35	69	.266
TOTAL	40.52	86	

Testing Interaction for Significance

$$F = \frac{.48}{.266} = 1.80 \quad n_1 = 8$$

$$n_2 = 69$$

F = 1.80 is less then .10